INBREEDING DEPRESSION IN METASEQUOIA

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EVER SINCE specimens of dawn redwood, *Metasequoia glyptostroboides* Hu & Cheng, grown from the Arnold Arboretum's 1948 seed introduction began to bear both microsporangiate and megasporangiate strobili (cones), there has been speculation as to whether the species would show inbreeding depression (U.S.D.A., 1974). Common symptoms of this phenomenon in conifers are reduced seed set and lowered vigor of progeny after selfing (Wright, 1976). This question is pertinent to *Metasequoia* because many of the trees now bearing cones are large, isolated specimens. Occasional seedlings have been raised from them, but viable seeds have been few (Wyman, 1968).

In the future it may be desirable to propagate the species from seed rather than from cuttings (as is usually done now), both for economy and to maintain genetic variability (Henkel, pers. comm.). It would be useful to identify the parent trees producing seed yielding the highest germination percentages and the most vigorous seedlings.

Because isolated specimens (400 m or more from the nearest other mature specimen) presumably have no means to produce fertile seed except through self-pollination or apomixis, it was possible to use a simple strategy to test for inbreeding depression and to identify the most desirable parent trees: comparison of seed and seedling characteristics from a sample of isolated trees with those from a sample of trees in mixed-clone groves where cross-pollination is likely. The characteristics chosen for testing were weight and germination percentage of cleaned seeds, rate of seedling growth, mortality, and evidence of abnormalities associated with reduced growth.

At the same time, optimum conditions of temperature and light for germination could also be determined by testing several different environments.

MATERIALS AND METHODS

CONES AND SEEDS. In November, 1981, 200 to 250 cones were collected from isolated trees (nos. 1–4, TABLE 1) and from trees in mixed-clone groves (nos. 5–9). Twenty cones from each tree were measured and weighed, and seeds of 10 cones from each tree were carefully removed and counted. The remaining cones were dried for a week at ambient indoor temperature and the seeds removed.

Seeds were cleaned in a General blower (manufactured by New Brunswick Sheet Metal Works, New Brunswick, New Jersey) at the U.S.D.A. Federal Seed Laboratory, New Brunswick, New Jersey. The upward velocity of the vertical air column that the seeds entered was adjusted by trial and error until nearly

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all filled seeds were accepted and nearly all empty ones were rejected. Each seed was examined in a diaphanoscope at magnifications of 7 to 10 × to check for presence of an embryo. From 30 to 300 seeds from each tree were counted, weighed, and placed on wet blotting paper in transparent plastic Petri dishes in a Pfeiffer Model 1C-D1 Daylight Seed Germinator. A 20°/30°C thermoperiod was used, with 16 hours at 20°C in darkness and 8 hours at 30°C with light. Germinants were counted every few days from the time germination began until it appeared to be complete.

Seeds of trees nos. 7 and 9, the two lots with the highest germination percentages, were then used to test five different germination environments: constant 15°C in darkness, constant 20°C with lights on 8 hours per day, 15°/25°C with lights on for 8 hours per day at 25°C, 20°/30°C as described above, and 20°/35°C with lights on for 8 hours per day at 35°C. Germinants were counted every few days until germination appeared complete.

To determine if all seeds containing live embryos were germinating under the test conditions (and by inference, whether stratification or chemical treatment could improve germination), samples from seedlots of trees nos. 5, 7, and 8 were cleaned in the blower at high air velocity, and then each sample was divided into two equal halves. One half was tetrazolium tested for live embryos, while the other was germination tested at 20°C. As a further check, seeds that had not germinated after 17 days were tetrazolium tested.

After testing of lots nos. 1 to 9 was complete, seeds from an additional grove tree and an additional isolated tree were received from Auburn, Alabama. These seedlots were added to the test because of their different geographic origin and their possibly different environmental conditions at pollination time, and in an attempt to obtain more "selfs."

SEEDLINGS. Seedlings from the three isolated trees that produced fertile seed were grown for comparison with seedlings from grove trees. Germinants to be tested were taken from the Petri dishes, potted in peat cubes, and replaced in the germinator for a week. They were then moved to a greenhouse at Cook College, New Brunswick, New Jersey, where they were grown under ambient daylight plus four hours of extra light supplied by cool-white fluorescent tubes between 10 P.M. and 2 A.M. nightly. Minimum night temperature was 18.3°C. When the seedlings had six to eight true leaves, they were moved to four-inch clay pots and transferred to another greenhouse with no supplemental light and a minimum night temperature of 15.6°C. They were watered daily, and every two weeks a nutrient solution (2.5 grams/liter of 20-19-18) was applied. Seedlings were measured and photographed on April 1, 1982.

STATISTICS. Statistical inferences from seed weights, germination, and comparative seedling heights are based on the nonparametric Wilcoxon rank-sum test (Lehmann, 1975).

RESULTS

CONES AND SEEDS. There were no significant differences between grove trees and isolated trees in cone length, cone weight, or number of seeds per cone

TABLE 1. **Comparison of cone and seed characteristics of isolated Metasequoia trees and those in mixed-clone groves.**

		Mean cone dimensions				Mean number of seeds				Germination percent	
Tree no.	Location	*Length* (cm)	R*	*Weight* (g)	R*	*Per cone*	R*	*Per pound*	R*		R*
Isolated trees											
1	New Brunswick, New Jersey	1.95	5	2.01	6	92	5	500,000	8	0.67	6
2	Princeton, New Jersey	1.92	6	1.93	7	88	7	1,400,000	9	0.33	7
3	Princeton, New Jersey	2.11	4	2.30	5	106	1	380,000	7	0.00	8.5
4	Princeton, New Jersey	1.90	7	2.48	4	76	8	350,000	6	0.00	8.5
	Mean	1.97		2.18		90		657,000		0.25	
	Rank sum		22		22		21		30†		30†
Grove trees											
5	Princeton, New Jersey	2.12	2.5	3.18	1	99	3	300,000	4	2.00	4
6	Princeton, New Jersey	1.60	8	1.90	8	74	9	280,000	3	3.70	3
7	Princeton, New Jersey	1.56	9	1.30	9	89	6	230,000	2	30.61	2
8	Locust Valley, New York	2.26	1	2.84	2.5	94	4	320,000	5	1.47	5
9	Locust Valley, New York	2.12	2.5	2.84	2.5	104	2	145,000	1	46.67	1
	Mean	1.93		2.41		92		255,000		16.89	
	Rank sum		23		23		24		10†		10†

*Rank order used in statistical analysis.
†$P < 0.01$.

TABLE 2. **Germination of Metasequoia seeds tested at different temperatures.**

TREE NO.	DAY	TEMPERATURE (°C) 15	20	15/25	20/30	20/35
5	9	0/16*	13/16	5/20	11/21	3/16
5	13	0/16	16/16	16/20	16/21	9/16
5	17	2/16	16/16	17/20	21/21	14/16
5	22	2/16	16/16	17/20	21/21	14/16
9	9	0/7	4/7	4/8	6/8	7/7
9	13	3/7	5/7	4/8	8/8	7/7
9	17	3/7	5/7	4/8	8/8	7/7
9	22	3/7	5/7	4/8	8/8	7/7

*Zero germinants of 16 seeds.

(TABLE 1). Differences in number of apparently filled seeds per pound and in percentage of these that germinated were highly significant ($P < 0.01$). Grove trees ranked 1 through 5 in both seed weight and germination percentage, with each tree holding identical ranks for both test criteria. The probability that all grove trees would surpass all isolated trees in either seed weight or germination percentage by pure chance is only 1 in 126.

Five grams of seed from the Alabama grove tree produced 155 apparently sound seeds, of which 112 germinated; 5 g from the isolated tree produced 13

TABLE 3. **Comparative heights of 13-week-old Metasequoia glyptostroboides seedlings from isolated specimens and trees in mixed-clone groves.**

TREE NO.	NO. OF SEEDLINGS	MEAN HEIGHT (cm)	RANK
ISOLATED TREES			
1	3*	9.0	9
2	10*	9.3	8
Rank sum			17†
GROVE TREES			
5	10	13.7	3
6	10	14.0	1.5
7	8	12.7	5
9	9	11.7	7
10‡	10	14.0	1.5
11‡	9	12.2	6
12‡	10	13.5	4
Rank sum			28†

*In addition to these 13 isolated-tree seedlings, six others are mentioned in the text: one from no. 1 and two from no. 2 (seven weeks older), and three from the Alabama isolated tree (four weeks younger; two dead).

†$P < 0.05$.

‡Trees 10, 11, and 12 are in a grove at Bailey Arboretum, Locust Valley, New York. Seeds were collected in December and were not included in the tests reported in TABLE 1.

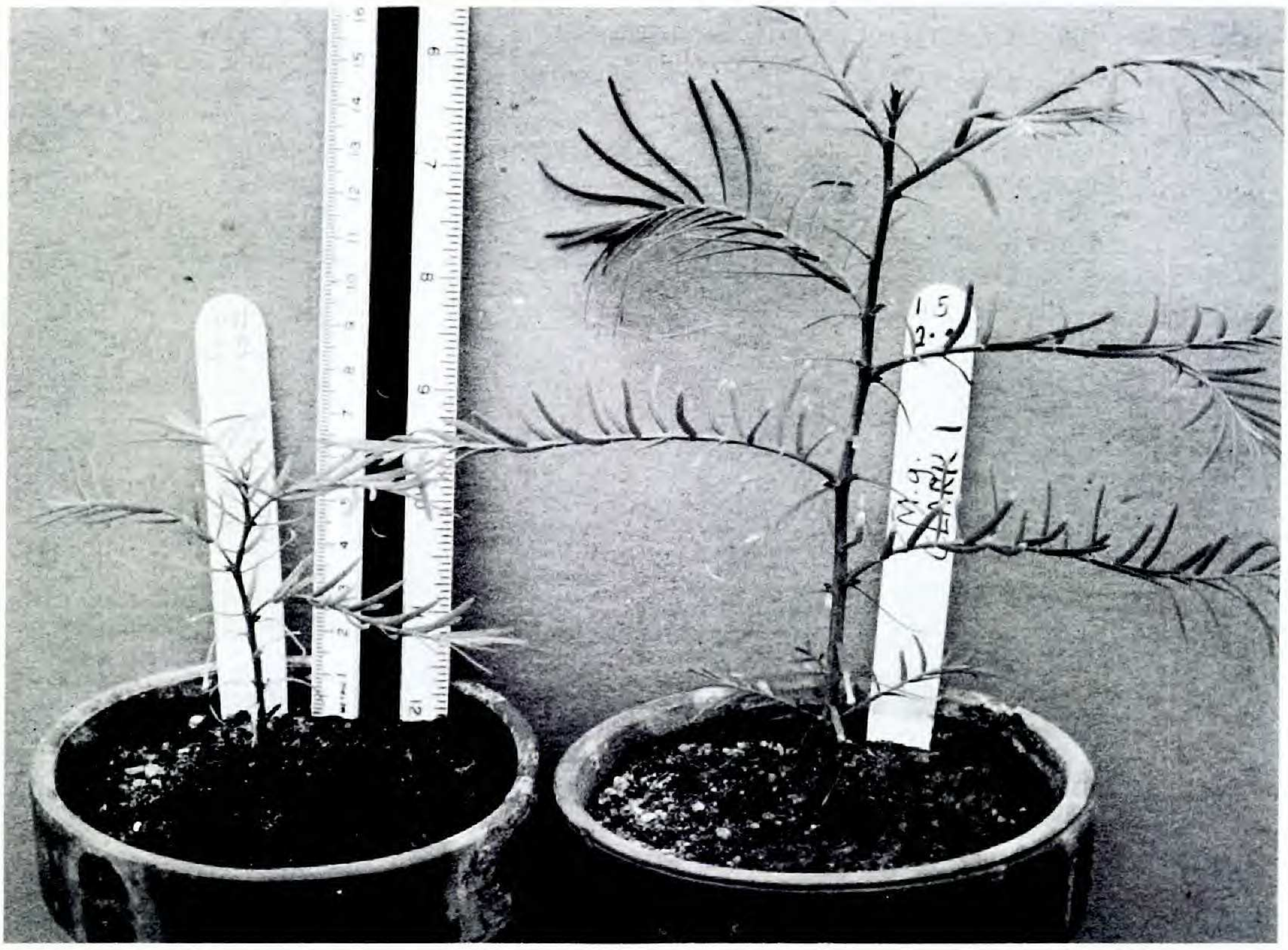

FIGURE 1. Thirteen-week-old *Metasequoia* seedlings from grove tree no. 5 (right) and isolated tree no. 1 (left).

apparently sound seeds, of which only 3 germinated. The order of difference in seed fertility between isolated and grove trees was the same in the Alabama trees as it had been in the New York and New Jersey ones.

Tetrazolium- and germination-testing of seedlots nos. 5, 7, and 8 gave similar results. Positive tetrazolium stains for the three lots, in order, were 5 of 7, 5 of 5, and 21 of 21; germination results were 6 of 7, 6 of 6, and 22 of 22. None of the seeds that failed to germinate gave a positive tetrazolium stain.

Among the five germination environments tested, constant 15°C was poorest, while constant 20°C, 15°/25°C, 20°/30°C, and 20°/35°C were satisfactory (TABLE 2). Visual inspection of germinants on day 13 showed those at 20°C and 20°/30°C to be larger than those at 15°/25°C, which in turn were much larger than those at 15°C and 20°/35°C.

SEEDLINGS. During the growth period, 3 of 19 isolated-tree seedlings died. One Alabama seedling was unable to shed its seed coat, and another had twisted cotyledons and failed to grow; 1 from lot no. 1 died after insecticide application. During this same period, only 1 of 128 grove-tree seedlings died. The difference in mortality was significant ($\chi^2 = 14.66$, $P < 0.01$).

Differences in height between the surviving isolated-tree and grove-tree seedlings were significant (TABLE 3, FIGURE 1). In addition to being slower growing, 3 of the 4 surviving seedlings of lot no. 1 and the sole surviving Alabama isolated-tree seedling were pale green in color, with some leaves having incipient fall coloration and tending to shrivel early. One seedling of lot no. 1 appeared normal, and all 11 of lot no. 2 appeared normal in color and habit, although they grew more slowly than grove-tree seedlings.

DISCUSSION

It seems reasonable to assume that our isolated trees' lighter seed weights and lower yield of fertile seed—and their progeny's higher seedling mortality, slower growth, cotyledon defects, and pale coloration—are all due to inbreeding depression. Apparently lots nos. 3 and 4 carry the greatest genetic load (recessive lethal and sublethal genes), because none of their seeds germinated. Lots nos. 1 and 15 seem to occupy an intermediate position: they produced five and three seedlings, respectively, of which all but one showed abnormal coloration or defective cotyledons. Lot no. 2 seems to carry the least load.

Conifers differ in their ability to "self," with some species showing greatly reduced seed set and others producing few to many viable seeds (Wright, 1976). Closely related to *Metasequoia, Sequoia* exhibits large clonal differences in relative germination of selfs and outcrosses (Libby *et al.*, 1981). The same is true of *Pseudotsuga menziesii*: self-pollinated ovules of some clones fail to produce viable seeds due to abortion of embryos (Orr-Ewing, 1957). Apparently, variation in the amount of genetic load carried by different trees causes some to be incapable of producing fertile self-pollinated seeds but allows others to produce a few viable seeds and occasional trees to self quite well. The amount of genetic load necessary to cause varying percentages of self-sterility has been calculated by several researchers (Bramlett & Popham, 1971; Bishir & Pepper, 1977).

Some of the variation in seed fertility among grove trees may be due to position in the grove with respect to availability of pollen from other clones. *Metasequoia* pollen is wingless and tends to clump together (U.S.D.A., 1974), and with this in mind I reexamined the spatial relationship of the grove trees to one another. Trees nos. 5 and 6 are at the uphill, northwestern (upwind at pollination time in March) end of their grove, while no. 7 is downhill, downwind, and surrounded by tall specimens bearing large quantities of microsporangiate strobili. In addition, the lower portions of the crowns of trees nos. 5 and 6 are suppressed, and megasporangiate strobili are present only in the tops, where pollen must be blown upward to reach them. Tree no. 8 stands in the open about 20 m from no. 9 and others.

It is clear from the comparison of germination- and tetrazolium-testing that no seed treatment (such as stratification) would have effected germination. To obtain good germination of *Metasequoia* seeds, one must collect cones from trees located advantageously for cross-pollination, then clean the seeds effectively by air-blower or equivalent technique to remove all empty ones. At the beginning of our experiment, it was difficult to distinguish filled and empty seeds in the diaphanoscope, and we ran the blower at medium speeds until we were able to judge its efficiency in retrospect by germination results. We then shifted to tetrazolium testing to determine which blower speed to use in cleaning seeds for the germination-environment tests and for the germination vs. tetrazolium comparison, and the resultant effects on germination percentage are obvious.

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